

EDGEWOOD

RESEARCH, DEVELOPMENT & ENGINEERING CENTER

U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-382

HD HYDROLYSIS/BIODEGRADATION TOXICOLOGY AND KINETICS

Steven P. Harvey
Linda L. Szafraniec
William T. Beaudry
Mark V. Haley
Thomas E. Rosso
Gerald P. Young

RESEARCH AND TECHNOLOGY DIRECTORATE

James P. Earley

SBR TECHNOLOGIES, INC.
South Bend, IN 46617

December 1996

DTIC STABILITY INFORMATION

Approved for public release; distribution is unlimited.



Aberdeen Proving Ground, MD 21010-5423

19970110 021

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 1996 December	3. REPORT TYPE AND DATES COVERED Final; 95 Oct - 96 Jun		
4. TITLE AND SUBTITLE HD Hydrolysis/Biodegradation Toxicology and Kinetics		5. FUNDING NUMBERS None		
6. AUTHOR(S) Harvey, Steven P.; Szafraniec, Linda L.; Beaudry, William T.; Haley, Mark V.; Rosso, Thomas E.; Young, Gerald P. (ERDEC); and Earley, James P. (SBR Technologies, Inc.)				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) DIR, ERDEC, ATTN: SCBRD-RTL, APG, MD 21010-5423 SBR Technologies, Inc., South Bend, IN 46617		8. PERFORMING ORGANIZATION REPORT NUMBER ERDEC-TR-382		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Project Manager, Chemical Demilitarization ATTN: AMCPM-CDO-C, APG, MD 21010		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES The information herein was presented at the 1996 ERDEC Scientific Conference on Chemical and Biological Defense, 19-22 November 1996, but will not be published in the Conference Proceedings.				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) One technology developed for potential use in the disposal of HD (2,2'-dichlorodiethyl sulfide) from the Aberdeen Proving Ground unitary chemical stockpile is hydrolysis followed by biodegradation. Stockpile HD (89.2% pure) was hydrolyzed in 90 °C water at a concentration of 3.8 wt %. The reaction was complete (HD undetectable at 200 ppb level), and the product typically contained 90-95% thiodiglycol. The complete product was amended with 1.5 g/L NH ₄ Cl, 0.28 g/L KH ₂ PO ₄ and trace mineral salts and fed to aerobic sequencing batch reactors, seeded with activated sludge. The efficiency of carbon removal in the bioreactors was typically >90%, and the effluent was nontoxic as determined by aquatic toxicity tests.				
14. SUBJECT TERMS HD Hydrolysis Biodegradation Soil Toxicology Kinetics			15. NUMBER OF PAGES 20	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

BLANK

PREFACE

The work described in this report was authorized under the Demilitarization Alternative Technologies Program funded by the Program Manager for Chemical Demilitarization. This work was started in October 1995 and completed in June 1996.

The use of trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Acknowledgments

The authors acknowledge Craig M. Frost (SBR Technologies, Incorporated) for his dedicated efforts operating the bioreactors; Dennis K. Rohrbaugh (ERDEC) for his GC/MS analysis of the stockpile HD; and Harry G. Taylor and George Roberts (ERDEC) for their expert assistance on the operation of the 35 gal chemical reactor.

BLANK

CONTENTS

1.	INTRODUCTION	7
2.	MATERIALS AND METHODS	7
2.1	HD Source and Analysis	7
2.2	Bioreactor Configuration	8
2.3	Bioreactor Conditions	8
2.4	Modified Wolin Salts Solution	8
2.5	Feedstock Preparation (HD Hydrolysis)	8
2.6	35 Gallon HD Hydrolysis Reactor	9
2.7	MICROTOX Assay	9
2.8	Gas Chromatography/Mass Spectroscopy	10
2.9	Nuclear Magnetic Resonance Spectroscopy	11
3.	RESULTS AND DISCUSSION	12
3.1	Hydrolysis	12
3.2	Biodegradation	14
3.3	Bioreactor Effluent Analysis	14
4.	CONCLUSIONS	16
	LITERATURE CITED	19

FIGURES

1.a	Reaction of 3.8% HD in water at 84-86 °C in a well-agitated system	13
1.b	CH-TG sulfonium ion	14
1.c	H2-TG sulfonium ion	14
2.	TOC and MICROTOX toxicity profiles versus reaction time for HD hydrolysate biodegradation process	16

BLANK

HD HYDROLYSIS/BIODEGRADATION TOXICOLOGY AND KINETICS

1. INTRODUCTION

HD is highly insoluble in aqueous solutions (Yang et al., 1988) and toxic to microorganisms because of its reactivity with enzymes and other proteins (Bush, 1946, pp. 431-439 for review). For these reasons HD is a poor candidate for direct biodegradation. However, HD does react with water to form hydrolysis products which are non-chlorinated, water-soluble and significantly detoxified. Consequently, these hydrolysis products are generally better candidates for biodegradation.

The mechanism of mustard hydrolysis at ambient temperature has been investigated previously (Bartlett and Swain, 1949, Helfrich and Reid, 1920, McManus et al., 1985, Yang et al., 1987a, Yang et al., 1988, Yang et al., 1987b.). It has been demonstrated that HD reacts through a series of sulfonium ion intermediates to produce thiodiglycol. However, additional ether or thioether products can also form depending on the actual conditions of the reaction. The rate of hydrolysis in water is limited by the rate of mass transfer because of the insolubility of HD in aqueous solution. Consequently, dissolution and reaction take place simultaneously (Yang et al., 1988).

Hydrolysis dechlorinates HD providing a significant detoxification. The resulting products are primarily biodegradable alcohols. The subsequent mineralization of these compounds can consequently be accomplished biologically at ambient temperature.

2. MATERIALS AND METHODS

2.1. HD Source and Analysis

HD was obtained directly from a one-ton storage container from the U.S. Army Aberdeen Proving Ground chemical stockpile where it had been stored for approximately 40 years. It was determined to be 89.2 area % pure by gas chromatography/mass spectrometry (GC/MS). The major impurity (4.7%) was (1,2-bis [2-chloroethylthioethane]), also known as compound Q or sesquimustard. The second most predominant impurity was dichloroethane (2.4%) which is probably formed as a thermal decomposition product from the HD dimer. The next most predominant products (2.0%) were the combined isomers of $C_6H_{12}Cl_2S$ which are believed to be thermal decomposition products of the \ and CH-TG sulfonium ions (HD hydrolysis products - Figures 1.b. and 1.c.) by way of reaction with chloride ion (Rohrbaugh et

al., 1989). The HD was used as received for all hydrolysis and biodegradation studies.

2.2. Bioreactor Configuration

New Brunswick BioFlo 3000 fermentors were fitted with 1-10 L vessels and used as Sequencing Batch Reactors (SBRs). Reactors were variously controlled either manually or automatically with New Brunswick Windows-based fermentation software.

2.3. Bioreactor Conditions

SBRs were operated on 24 hour cycles. Seed cultures were obtained from activated sludge (Back River Wastewater Treatment Plant, Baltimore, MD). Initial mixed liquor suspended solids (MLSS) levels were approximately 2800 ppm. Modified Wolin Salts solution (10 ml/L) was added to provide inorganic micronutrients along with 1445 mg/L NH_4Cl and 278 mg/L KH_2PO_4 as nitrogen, potassium and phosphate sources. Hydrolyzed HD was provided as the sole source of carbon and sulfur. Operating pH was maintained between 6.5 and 8.5, primarily by the addition of 15 g/L of NaHCO_3 as a buffer. Also, NaOH was occasionally added on demand through a pH controller when the pH dropped below 6.5. Mineralization of sulfur-containing compounds such as TDG causes acid production when the sulfur is converted to sulfate which must be charge-balanced with two cations.

2.4. Modified Wolin Salts Solution

Modified Wolin Salts Solution was prepared 100x as follows: 3.0 g/L nitrilotriacetic acid, 6.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L MnSO_4 , 0.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g/L H_3BO_3 , 0.01 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.01 g/L $\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

2.5. Feedstock Preparation (HD Hydrolysis)

HD for all biodegradation studies was obtained directly from the U.S. Chemical Stockpile at Aberdeen Proving Ground, MD and was used exactly as received from the one ton storage container. It was routinely hydrolyzed for two hours at 90° C at a concentration of 1% (vol/vol) in either 0.67% NaOH (wt/vol) or tap water. A 3 L roundbottom, water-jacketed flask agitated with a motor-driven Teflon paddle was used for the reactions. HD was added over a period of 30 minutes from a separatory funnel at the top of the flask. In addition to the expected organic products a solid precipitate was formed. Electron microscopy analysis indicated the material was comprised primarily of iron complexes. The iron was presumably derived from the steel container in which the HD had been stored for approximately 40 years. The mass of

the precipitated material was equivalent to less than 1% of the mass of the starting HD. The HD/water hydrolysis reaction also produced a slightly smaller quantity of a similar material after pH neutralization with NaOH. Hydrolysate solutions were routinely analyzed for HD by extracting 50 ml of hydrolysate with 1 ml CHCl_3 followed by GC/MS analysis of the extract to a detection limit of 160 parts per billion (ppb). The U.S. Army drinking water standard for HD is 200 ppb. All solutions which were hydrolyzed and analyzed by these techniques were found to contain no detectable HD at the 160 ppb level. Solutions were also routinely analyzed by NMR to determine the relative area percent of TDG and ethers and also for sulfonium ions.

2.6. 35 Gallon HD Hydrolysis Reactor

The HD hydrolysis reaction profile was obtained from a 35 gallon batch reaction conducted in a modified 55-gallon stainless steel drum 30.5" high by 22.75" wide and fitted with a 5/16" thick polypropylene drum-liner. A nickel alloy coil circulating hot water was used to maintain the temperature at 84-86 C. Agitation (350 rpm) was provided by two impellers: a 6" Rushton turbine mounted one impeller diameter above the bottom of the drum to provide shearing action and a 7" pitched-blade axial impeller mounted at the mid-point of the impeller shaft to provide top to bottom circulation in the vessel. The impellers and shafts were coated with polyvinylidene fluoride polymers (PVDF) to prevent corrosion by the acidic hydrolysate solution. The reactor also contained four 34" long angle baffles consisting of 2" x 1.5" nickel alloy running vertically on the tank wall. All HD was added over a four minute period prior to the start of agitation. Sampling was done manually with a 50 ml syringe beginning 42 seconds after the start of agitation and continuing at increasing intervals up to 120 min. Samples were extracted twice, each time using 1/10 volume of chloroform. The organic layer was analyzed for HD by GC/MS and the aqueous layer was analyzed by NMR for sulfonium ions and thiodiglycol.

2.7. MICROTOX Assay

The MICROTOX Bioassay exposes a bioluminescent marine bacterium to a toxicant and measures the change in light output as the means of determining effects on the organisms [5]. The bacterium *Vibrio fischeri* is cultured by Microbics Corporation, and shipped in lyophilized form. The bacterium (stored frozen) is reconstituted immediately before testing. Each bioassay uses less than 3 ml of toxicant and is performed in a temperature controlled photometer. Light output readings are measured after 5 and 15 minutes in to the exposure. The change in light output is compared to the light output of the control and the EC-50 is

calculated using MICROTOX statistical software.

The assay is performed in glass cuvettes which incubate in temperature controlled wells of a photometer. Thirty test wells are available, so several assays can be performed simultaneously. The assay must have a minimum of four dilutions exhibit a dose-response for optimum accuracy in predicting an EC50.

The salt concentration of neutralized HD was approximately 2 %, therefore salt adjustment to the samples were not needed. Adjustments to pH were done before testing if needed using 10 % NaOH. Due to interference caused by suspended particles, the samples were allowed to settle and the supernatant decanted and used in testing.

The assay was set up using 100 % sample with 2 replicates of 5 treatment groups (1:2 serial dilution) and one control. The 100% treatment group cuvettes received 2 ml of sample, then two-fold serial dilutions were made by removing 1 ml of sample from the 100% treatment group cuvettes and then added to the adjacent 50% treatment group. The 50% treatment group was mixed and 1 ml removed and added to the 25% treatment group. This procedure was continued for each dilution until reaching the control, where the 1 ml sample from the final dilution was disposed of. The same procedure was followed using phenol as a reference toxicant. The results from the phenol assays are used to monitor the response of the organisms.

Bacteria were transferred to the cuvettes in 10 ml aliquots and mixed. The addition of bacteria is referred to as time zero. Five minutes after time zero, the control cuvette was used to calibrate the photometer to 100% light output. The control and test cuvettes were returned to the incubator and re-measured at 15 minutes. Data were analyzed with the MICROTOX Test Protocol software to determine the EC-50 for both time intervals.

If the toxicity of the sample was too high for determining a reliable EC50, it was diluted before being subjected to the 1:2 serial dilution protocol.

2.8. Gas Chromatography/Mass Spectroscopy

GC/MS analyses of HD from 35 gallon reactor samples were conducted using a Hewlett-Packard 5890 GC with 5970 mass selective detector. HD was quantitated using selected ion monitoring for HD ions 109, 111, 158, and 160. The parameters

for the GC consist of an initial temperature of 70° C with a one min hold followed by a 4° C/min ramp to 110° C then 25° C/min ramp to a final temperature of 250° C with a five min hold. The injector was held at 250° C with 50 ml/min split flow which was turned on at 0.6 min. A four point calibration curve of HD in hexane was generated prior to analysis of sample extracts. The calibrations correlation coefficient was 0.998 and the detection limit was 1 ppm.

2.9. Nuclear Magnetic Resonance Spectroscopy

NMR analyses were conducted using a Varian Unity Plus 400 Fourier Transform (FT) NMR spectrometer which operates at 400 MHz for ^1H observation and at 100 MHz for ^{13}C observation. All spectra were obtained at probe temperature (22 +/- 1° C) with double precision data accumulation. Samples were provided in CDCl_3 , D_2O or water. All samples run in CDCl_3 were referenced to internal tetramethylsilane (TMS) using the CHCl_3 resonance ($\delta^1\text{H}$, 7.24; $\delta^{13}\text{C}$, 77.2) as a secondary reference. ^1H spectra in D_2O and water were referenced to external sodium 3-trimethylsilylpropionate-2,2,3,4- d_4 (TSP) in D_2O . Quantitative data were obtained by digital integration of peak areas.

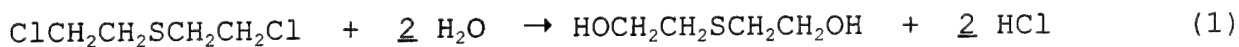
Survey ^1H spectra were run for product identification using a sweep width of 8000 Hz (20 ppm), a pulse width of at least 12 microseconds (30 degrees), an acquisition time of 2-4 seconds and a pulse delay of 2-4 seconds. Corresponding ^{13}C spectra were acquired using a sweep width of at least 25000 Hz (250 ppm), an acquisition time of 1.6 seconds, and a pulse delay of 2-3 seconds. Spectra were accumulated until the desired signal-to-noise ratio was achieved. Pre-saturation of the large H_2O peak was often used (standard Varian software) to minimize this resonance in the spectrum.

Spectra obtained for kinetic runs were acquired with fixed gain and integral values using the absolute intensity (AI) mode of the spectrometer. Each set of samples from a reaction were analyzed under identical operating conditions so that the integral area from different samples from the set could be directly compared. For ^1H spectra, 16 transients were collected using a sweep width of 8000 Hz (20 ppm), an acquisition time of 2.0 seconds, a pulse width of 7 microseconds (21 degrees) and a pulse delay of 2 seconds. For ^{13}C spectra, 64 transients were collected using a sweep width of 25000 Hz, an acquisition time of 2 seconds, a pulse width of 12 microseconds (90 degrees), and a pulse delay of 2.5 seconds. ^1H data obtained in CDCl_3 were normalized to the residual CHCl_3 resonance in the solvent. Data obtained in water or D_2O were not normalized.

3. RESULTS AND DISCUSSION

3.1. Hydrolysis

The essential overall HD hydrolysis reaction is described by equation (1):



In actual practice, the yield of thiodiglycol is dependent on the pH of the reaction. Reactions conducted in water alone yield a relatively high percentage of thiodiglycol (90-95 area % by NMR), whereas reactions conducted in an excess of aqueous NaOH (simultaneous hydrolysis and neutralization) typically yield around 65 area % thiodiglycol, with the remainder of the compounds comprised primarily of ether-alcohols (Harvey et al., in press). Thiodiglycol is biodegradable (Beaudry et al., 1994), consistent with the finding that the water-hydrolyzed product was more biodegradable (>90% total organic carbon removal) than the aqueous NaOH-hydrolyzed product (~65% total organic carbon removal). Based on these observations, all hydrolysis reactions for these studies were conducted in water. NaOH was used for neutralization of the HCl subsequent to the hydrolysis reaction.

Results of experiments on the effects of temperature and HD concentration on the rate and products of the hydrolysis reaction have been described previously (Harvey et al., in press). Briefly, the reaction proceeds relatively slowly at 30° C and is accelerated approximately 28-fold by an increase in temperature to 70° C (rates were described in relative terms due to the mass-transfer limited nature of the reaction which makes the rate dependent on agitation). Thiodiglycol is the main hydrolysis product; various sulfonium ions are intermediates in the reaction (Yang et al., 1987a).

Figure 1 illustrates the progress of the HD hydrolysis reaction in water as followed by sampling and analysis from a 35 gallon reaction conducted at 84-86° C. Configuration, operation and sampling of the reactor are described in 3.2. METHODS above. Results in Figure 1.a show the decrease in HD concentration (plotted logarithmically against the left axis) and the increase in thiodiglycol concentration (plotted linearly against the right axis) as a function of time. The HD concentration decreases from 38,000 ppm to less than 0.2 ppm (the U.S. Army drinking water standard for HD) in less than 20 minutes, corresponding to a half-life of approximately 30 seconds. The concentrations of two sulfonium ion intermediates, CH-TG and H-2TG, are also plotted as

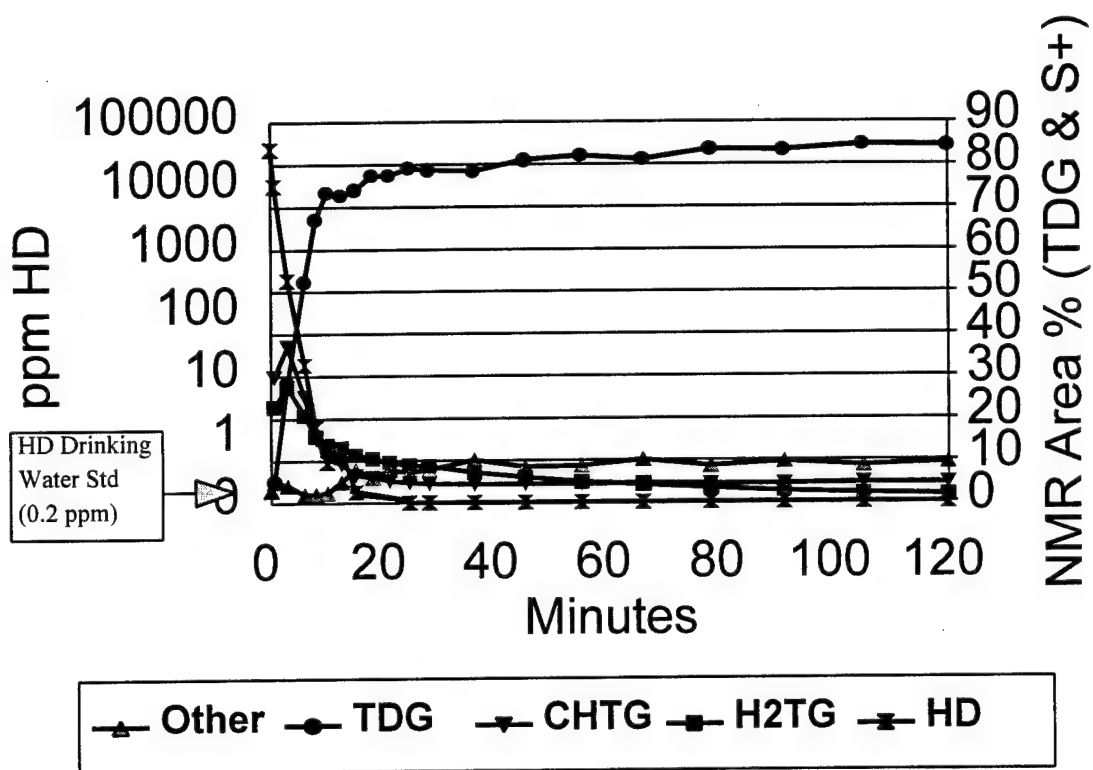


Figure 1. Reaction of 3.8% HD in water at 84-86° C in a well-agitated system.

determined by NMR analysis. The structures of these sulfonium ions are illustrated in Figure 1.b and 1.c. The half-life of the H-2TG sulfonium ion was measured at 38 minutes under these conditions. Not shown on this graph are the concentrations of the chlorinated sulfonium ion $(\text{HOCH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl}$ which was detected in the 42 second (10.6 area %) and three minute (2.0 area %) reaction samples.

At the end of the reaction, NMR analysis showed thiodiglycol to comprise 84.4 area % of the product, CH-TG 4.5 area %, H-2TG 1.7 area % and other unidentified compounds 9.4 area %. HD was undetectable (detection limit <150 ppb) throughout the last 100 minutes of the reaction.

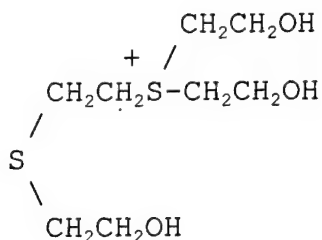


Figure 1.b: CH-TG sulfonium ion.

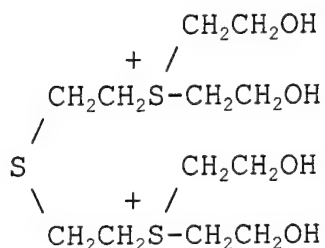


Figure 1.c: H2-TG sulfonium ion

3.2. Biodegradation

In order to meet international treaty requirements (Report of the conference on disarmament to the General Assembly of the United Nations, 1992), it is necessary to not only detoxify chemical agents but also to destroy them irreversibly. In order to address this issue of final disposal, biodegradation of the hydrolyzed HD products was studied in a sequencing batch reactor (SBR). SBRs offer several important operational advantages for the treatment of chemical wastes (Irvine and Ketchum, 1989 for review). First, they are efficient. The SBR models as a continuous flow stirred tank reactor (CFSTR) followed by a plug flow reactor, offering the ideal volumetric reactor configuration for unsteady state activated sludge systems. Second, they operate in the batch mode which not only offers a kinetic advantage over conventional CFSTRs (therefore smaller size) but, for hazardous waste treatment, they permit batch analysis and toxicity testing prior to discharge. Third, they use a single tank for both treatment and settling; therefore, no secondary clarifier is required. Finally, they are robust and flexible. SBRs are intentionally operated over a range of substrate concentrations, pH conditions and oxygen concentrations, thereby allowing selection of a very diverse and robust population of microorganisms. Operational strategies can also be varied to accomplish carbon, nitrogen and/or phosphorus removal.

Subsequent to hydrolysis, NaOH (2.1 moles per mole of HD) was added to the hydrolysate to neutralize the HCl. The final pH of the hydrolysate was approximately 11. This was diluted three-fold with water and amended with 1.5 g/L NH_4Cl , 0.28 g/L KH_2PO_4 and 10 ml/L Wolin Salts and fed to aerobic Sequencing Batch Reactors (SBRs) seeded with activated sludge from the Back River Wastewater Treatment Plant in Baltimore, MD. NaOH addition caused dissolved iron to precipitate out of solution; the precipitate was fed to the SBRs as a slurry along with the dissolved organics and salts.

SBRs are advantageous for the treatment of chemical wastes since their batch operation permits analysis of effluent prior to discharge. SBRs also provide the kinetic advantages of a continuous flow stirred tank reactor followed by a plug flow reactor; the ideal volumetric reactor configuration for unsteady state activated sludge systems. Reactors were typically operated on a 10 day hydraulic residence time with a four hour aerobic fill period, 18 hour react (aeration) period, one hour settle and one hour idle/draw. Mixed liquor suspended solids (MLSS) concentrations ranged between approximately three and five grams per liter.

3.3. Bioreactor Effluent Analysis

The bioreactor effluent analysis showed an average removal efficiency of 99.6% for thiodiglycol and 90.6% for total organic carbon (TOC). Figure 2 shows the TOC profile and the results of MICROTOX luminescent marine bacteria toxicity testing versus reaction (aeration) time. The MICROTOX data are presented as EC_{50} values. EC_{50} is defined as the effective concentration of material which decreases luminescence (viability) of the test organisms by 50%. The toxicity initially decreases and the TOC increases during the fill period when feed is being added to the reactor. As the fill is completed and biodegradation continues, the TOC drops and the toxicity decreases (i.e. the EC_{50} value increases). Although organic carbon removal is essentially complete within a 22 hour react period (a 10 day hydraulic residence time as the reactors are typically operated), the decrease in toxicity of the bioreactor effluent continues for approximately twice that long (up to 48 hours react which would be equivalent to a 20 day hydraulic residence time). Although the exact cause of the discontinuity between TOC removal and toxicity reduction is not known, a simple explanation would be that one or more compounds of relatively minor abundance account for a significant portion of the detectable toxicity and that these compounds are biodegraded at a rate which is slow relative to that of the major portion of the organic carbon. Not shown in

Figure 2 is the toxicity of the starting feed material which had an EC_{50} of approximately 2%. The final effluent, biodegraded for the equivalent of a 20 day hydraulic residence time (48 hours reaction with a 10 day hydraulic residence time) at the above conditions, was completely non-toxic to luminescent marine bacteria.

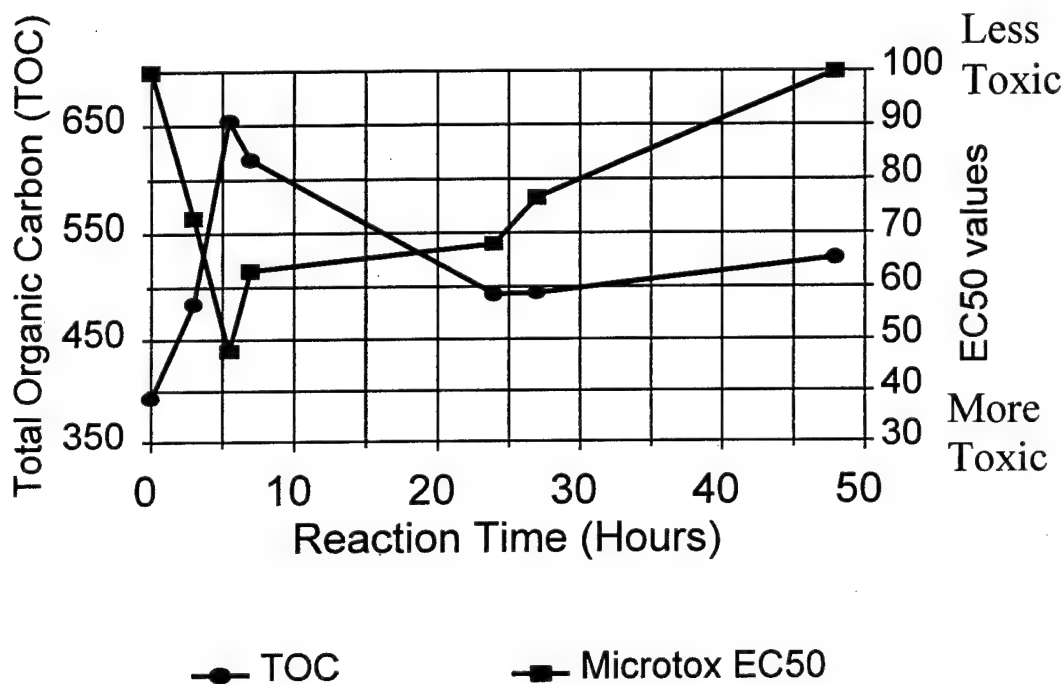


Figure 2. TOC and MICROTOX toxicity profiles versus reaction time for HD hydrolysate biodegradation process.

4. CONCLUSIONS

HD, at a 3.8% loading in water, in a well-mixed reactor at 84-86° C, reacts with a half-life of approximately 30 seconds to a concentration below 200 ppb within 20 minutes. The half-life of the H-2TG sulfonium ion under these same conditions is 38 minutes. The hydrolysate, as analyzed by NMR, is comprised primarily of thiodiglycol with small amounts of H-2TG and CH-TG sulfonium ions.

When the hydrolysate was diluted with three volumes of

water, amended with 1.5 g/L NH_4Cl , 0.28 g/L KH_2PO_4 and mineral salts and fed to aerobic SBRs seeded with activated sludge (MLSS = three to five g/L), greater than 99% of the thiodiglycol and greater than 90% of the total organic carbon was removed (converted to CO_2 or biomass). MICROTOX studies demonstrated complete detoxification with a react time of 48 hours although TOC removal was essentially complete after 24 hours react time.

BLANK

LITERATURE CITED

1. Bartlett, P.D. and Swain, C.G. 1949, Kinetics of Hydrolysis and Displacement Reactions of β, β' -Dichlorodiethyl Sulfide (Mustard Gas) and of β -chloro- β' -Hydroxydiethyl Sulfide (Mustard Chlorohydrin), J. Am. Chem. Soc. 71, 1406-1415.
2. Beaudry, W.T., Bossle, P.C., Harvey, S.P., Kolakowski, J.E., Procell, L.R., Rohrbaugh, D.K., Sorrick, D.C., Stroup, A.N., Szafraniec, L.L. and Yang, Y.C. 1994. Neutralization of Munitions-Grade HD to Biodegradable Components. in 19th Army Science Conference Proceedings, Assuring the Competitive Edge. Army Science Board, eds. Vol. 4 pp. 1497-1504. Washington D.C.
3. Bush, V. 1946. Summary of Technical Report of Division 9, National Defense Research Committee. Washington, D.C.: Columbia University Press.
4. Harvey, S.P., Szafraniec, L.L., Beaudry, W.T., Earley, J.P. and Irvine, R.L. Neutralization and Biodegradation of Sulfur Mustard, in S.K. Sikdar and R.L. Irvine (eds.), Bioremediation: Principles and Practice Created, Vol. II: Biodegradation Technology Developments, Technomic Publishing Inc. In press.
5. Helfrich, O.B. and Reid, E.E. (1920). Reactions and Derivatives of β, β' -Dichloro-ethyl Sulfide, J. Am. Chem. Soc. 42, 1208-1232.
6. Irvine, R.L. and L.H. Ketchum, Jr. (1989). Sequencing Batch Reactors for Biological Wastewater Treatment, CRC Critical Reviews in Environmental Control, Vol. 18, Issue 4, 255-294.
7. McManus, S.P., Neamati-Mazrach, N., Hovanes, B.A., Paley, M.S. and Harris, J.M. 1985, Hydrolysis of Mustard Derivatives. Failure of the Raber-Harris probe in predicting nucleophilic assistance. J. Am. Chem. Soc. 107: 3393-3395.
8. Rohrbaugh, D.K., Yang, Y.-C., and Ward, J.R. 1989, The characterization of sulfonium chlorides by gas chromatography/mass spectrometry and the degradation of 2-chloroethyl sulfide derivatives. *Phosphorus, Sulfur and Silicon* 44:17-25.
9. Yang, Y.-C., Szafraniec, L.L., Beaudry, W.T. and Ward, J.R. 1987a, Direct NMR Measurements of Sulfonium Chlorides

Produced from the Hydrolyses of 2-Chloroethyl Sulfides. J. Org. Chem. 52, 1637-1638.

10. Yang, Y-C., Szafraniec, L.L., Beaudry, W.T. and Ward, J.R. 1988, Kinetics and Mechanism of the Hydrolysis of 2-Chloroethyl Sulfides. J. Org. Chem. 53, 3293-3297.

11. Yang, Y.C., Ward, J.R., Wilson, R.B., Burrows, W. and Winterle, J.S. 1987b, On the Activation Energy for the Hydrolysis of Bis-(2-chloroethylethyl) Sulfide. II. Thermochim. Acta. 114, 313-317.